Effects of Coenzyme Q10 on the Heart Ultrastructure and Nitric Oxide Synthase during Hyperthyroidism

Fusun Oztay[1], Bulent Ergin[1], Savas Ustunova[1], Huriye Balci[2], Aysegul Kapucu[1], Metin Caner[3], and Cihan Demirci[1]

[1]*Department of Biology, Faculty of Science*
Istanbul University Vezneciler
[2]*Fikret Biyal Research Laboratory, Cerrahpasa Medicine Faculty*
Istanbul University
and
[3]*Department of Internal Medicine, Cerrahpasa Medicine Faculty*
Istanbul University
Istanbul-Turkey

Abstract

Coenzyme Q10 is an important component of mitochondrial electron transport chain and antioxidant. Hyperthyroidism manifests hyperdynamic circulation with increased cardiac output, increased heart rate and decreased peripheral resistance. The heart is also under the oxidative stress in the hyperthyroidism. The aim of this study was to examine both how the coenzyme Q10 can affect heart ultrastructure in the hyperthyroidism and how the relationship between nitric oxide synthase (NOS) and heart damage and coenzyme Q10. Swiss Black C57 mice received 5 mg/kg L-thyroxine. Coenzyme Q10 (1.5 mg/kg) and L-thyroxine together was given to second group mice. Coenzyme Q10 and serum physiologic were applied to another two groups, respectively. All treatments were performed daily for 15 days by gavage. Free triiodothyronine and thyroxine were increased in two groups given L-thyroxine; thyroid-stimulating hormone level did not change. Hyperthyroid heart showed an increased endothelial NOS (eNOS) and inducible NOS (iNOS) immunoreactivity in the tissue. Coenzyme Q10 administration decreased these NOS immunoreactivities in the hyperthyroid animals. Cardiomyocytes of the hyperthyroid animals was characterized by abnormal shape and invaginated nuclei, and degenerative giant mitochondria. Desmosome plaques reduced in density. In hyperthyroid mice given coenzyme Q10, the structural disorganization and mitochondrial damage regressed. However, hearts of healthy mice given coenzyme Q10 displayed normal ultrastructure, except for increased mitochondria and some of them were partially damaged. Coenzyme Q10 increased the glycogen in the cardiomyocytes. In conclusion, coenzyme Q10 administration can prevent the ultrastructural disorganization and decrease the iNOS and eNOS increment in the hyperthyroid heart.

Key Words: hyperthyroid heart, nitric oxide synthase, ultrastructure, CoQ10, mice

Introduction

Hyperthyroidism manifests hyperdynamic circulation with increased cardiac output, increased heart rate and decreased peripheral resistance (4, 12, 13). The heart is also under the oxidative stress in the hyperthyroidism (2, 8). Ultrastructural studies reported structural damage, such as particularly mitochondrial damage as well as the development of destructive changes in the contractile apparatus of the myocytes in hyperthyroid heart (7, 9, 24).

Nitric oxide (NO) is a gas molecule synthesized

Corresponding author: Dr. Füsun Öztay, Istanbul University, Faculty of Science, Department of Biology, 34459 Venzeciler, Istanbul, Turkey. Tel: 00 90 212 455 57 00; Fax: 00 90 212 528 05 27; E-mail: fusunoztay@yahoo.com
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from L-arginine by the catalytic reaction of different isoforms of nitric oxide synthases. In mammals, there are three known members of them: the neuronal isoform (nNOS), the inducible isoform (iNOS), and the endothelial isoform (eNOS)(22). Both nNOS and eNOS are constitutively expressed in myocardium. The eNOS is mainly expressed in the heart, except during cardiac pathology where cytokine-induced iNOS expression may dominate (1). The eNOS is present in endothelial cells, endocardial cells, and cardiomyocytes. The cardiac eNOS involve in regulation of vascular resistance, myocardial perfusion and heart contractility (11, 12). The presence of iNOS noted in endothelial cells, vascular smooth muscle cells, macrophages and cardiomyocytes in disease states of heart. Disease heart exhibited elevated iNOS expression, depending on inducing oxidative stress (25, 30).

Coenzyme Q10 (CoQ10) is the electron transporter in the mitochondrial respiratory chain. CoQ10 is widely used as a therapeutic agent in pathology, as it could both stimulate oxidative phosphorylation by complementing any defects in mitochondrial respiration and protect against oxidative damage (5, 16). There is a significant inverse correlation between CoQ10 and thyroid hormones levels. CoQ10 circulating levels decreased in hyperthyroidism (23, 31). The cardio-protective effect of exogenous CoQ10 could be attributed to the preservation of mitochondrial function during pathological state as evidence proven by improved FADH-dependent oxidation (5).

Therefore, the purposes of this study were to examine relationship between the hyperthyroidism and NO and to detect how the CoQ10 administration to hyperthyroid mice can affect both the ultrastructural alterations of hyperthyroid heart and its immuno-histochemical reactivity for eNOS and iNOS. Moreover, we would like to investigate a probable correlation between the NO and CoQ10 in hyperthyroid mice heart.

Materials and Methods

Animals

Swiss Black C57 male mice (3-4 months old) were used in the present study. Mice were kept in standard housing conditions in an acclimatized room (22 ± 3°C) and fed with pellet chow and water ad libitum. The experiments were reviewed and approved by the Institute's Animal Care and Use Committee of the University of Istanbul.

Experiment

The mice were divided into four groups (n = 5 per group): mice received serum physiologic (Group I, control group), 5 mg/kg L-thyroxine [(Tefor, 0,4 mg/kg, Organon, Postbus, Netherland) for hyperthyroidism (Group II)], the same dose L-thyroxine together with 1.5 mg/kg [Solgar Istanbul, Turkey, (Group III)] and the same dose CoQ10 dissolved in serum physiologic (Group IV) daily for 15 days by gavage. All mice were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal 120 mg/kg) at the end of the 15th day and sacrificed.

Biochemical Assays

Blood samples were obtained from the hearts of anesthetized mice on the 15th day. The thyroid status of mice was confirmed by measurement of serum free triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH) levels by radioimmunoassay (Free T_3, T_4: competitive, analogy-based immunoassay; TSH: third generation chemiluminescent immunometric assay, Kit immulite DPC; Immulite 2500 Analyzer DPC, Los Angeles, CA, USA). The results were expressed as Arithmetic mean ± standard deviations. The statistical significance was assessed by Kruskal-Wallis One-Way ANOVA and multiple comparisons Post Hoc Dunn test, using Unistat version 5.0 statistical package.

Histological Assays

The dissected mouse hearts were fixed with 10% neutral formalin for 24 h for light microscopic studies and subsequently a routine paraffin embedding method was used. Heart sections of 4 μm thickness stained with hematoxylin-eosin (HE) were examined under a light microscope. For ultrastructural investigations, the ventricle pieces were fixed by 2% glutaraldehyde and 1% osmiumtetraoxide, rinsed in cacodylate buffer (pH 7.4), quickly dehydrated in ethanol and embedded in Epon 812. The thin sections were stained with uranyl acetate-lead citrate and examined under JEOL-1011 electron microscope.

Immunohistochemistry

The mouse hearts fixed with 10% neutral formalin for 24 h were used for a streptavidin-biotin complex (StrepABC) immunohistochemical method. The sections of 4 μm thicknesses were deparaffinized in xylene and rehydrated in a descending series of ethanol. The specimens were subjected to antigen retrieval in citrate buffered solution (pH 6.0) for 10 min by microwave. Endogenous peroxidase was eliminated by incubation in 10% H_2O_2 in phosphate-buffered saline (PBS; 0.01 M; pH: 7.4) for 10 min. After being washed, the specimens were blocked in ready-use normal goat serum for 20 min at room temperature (RT). The sections were incubated with the rabbit

Table 1. Comparisons of biochemical parameters for serum T_3, T_4 and TSH among experimental groups

	Control Mice n = 4	Mice Received L-Thyroxine n = 4	Mice Received L-Thyroxine plus CoQ10 n = 4	Mice Received CoQ10 n = 5	Test Results
Free T_3 (ng/ml)	1.00 ± 0.00 (1.00)[a]	2.99 ± 0.25 (2.99)*	2.93 ± 0.34 (2.93)*	1.03 ± 0.19 (1.09)	P = 0.0037 Significant
Free T_4 (mg/dl)	0.85 ± 0.00 (0.85)	6.00 ± 0.00 (6.00)**	6.00 ± 0.00 (6.00)**	1.04 ± 0.19 (1.09)	P = 0.0027 Significant
TSH (mIU/ml)	< 0.004	< 0.004	< 0.004	< 0.004	Non-Significant

[a] Mean ± Standard Deviation (Median)
*P < 0.05 compared to control mice
**P < 0.01 compared to control mice

anti-mouse eNOS (Neomarker, RB-1711-p) and iNOS (Neomarker, RB-1605-p) antibodies in a dilution of 1:100 1 h at RT. Briefly, the sections were processed by StrepABC procedure following the manufacturer's instructions (by using a goat anti-rabbit IgG, Labvision, TR-060-BN and TR-060-HR, Suffolk, UK). The peroxidase activity was demonstrated by AEC (3-amino-9-ethyl carbazole) substrate kit (Labvision, TA-004-HAC). The sections were rinsed in PBS. Primary antisera were diluted in antibody diluent (Labvision, TA-125-UD). Control procedures were performed on adjacent sections of the same tissues. No immunolabelling was detected when the primary antibody was omitted or replaced with either PBS or isotype rabbit antiserum instead of primary antiserum (Zymed, San Francisco, CA, USA, 08-6199).

Results

Biochemical Results

Serum free T_3 and T_4 were significantly increased in mice given L-thyroxine and mice given L-thyroxine plus CoQ10 (Table 1). These results indicated that both of them received L-thyroxine are hyperthyroid.

Histological Results

The myocardium of control mice and mice given CoQ10 showed a typical structure of myocardium (Figs. 1a and 1d). However, hyperthyroid heart was generally formed by elongated cardiomyocytes that was not joined to one another in linear array and possessed with elongated nuclei (Fig. 1b). In hyperthyroid heart given CoQ10, damage was less intensive and occurred partially (Fig. 1c). In addition, healthy hearts and hyperthyroid hearts of mice given CoQ10 displayed hyperaemia. Ultrastructurally, a great degree of structural damage was noticed in hyperthyroid heart, when compared with controls (Figs. 2a, 2b, and 2c). Hyperthyroid heart cardiomyocytes were characterized by abnormal shaped and invaginated nuclei, and degenerative giant mitochondria. Furthermore, the disruption of arrays of myofibrils and numerous mitochondria was observed. They were giant and swollen with disrupted cristae, between these myofibrils (Fig. 2c). In heart of hyperthyroid mice given CoQ10, longitudally arrayed myofibrils improved. Additionally, mitochondrial damage partially regressed despite of the presence of the degenerative changes in some of mitochondria (Fig. 2d). On the other hand, the hearts of healthy mice given CoQ10 had normal ultrastructure except for mitochondria increased in number and slightly mitochondrial damage in some of them (Fig. 2e). Glycogen particles were abundant in all experimental groups, when compared to control group. But, the highest presence of glycogen particles was in the hearts of healthy mice given CoQ10.

Hyperthyroid hearts also showed disorganization of intercellular junctions and desmosomes with attachment plaque reduced in density, when compared to controls (Figs. 3a and 3b). CoQ10 administration to hyperthyroid animals reversed structure of intercellular junctions to control images of junctions (Fig. 3c). Furthermore, structure of intercellular junctions was quite similar to those of the controls in healthy hearts given CoQ10 (Fig. 3d).

Immunohistochemical Results

In the control animals, eNOS-immunoreactivity (IR) was present in both endothelial cells and cardiomyocytes, while iNOS-IR was slightly detected in the cardiomyocytes (Figs. 4a and 5a). Notably, elevated

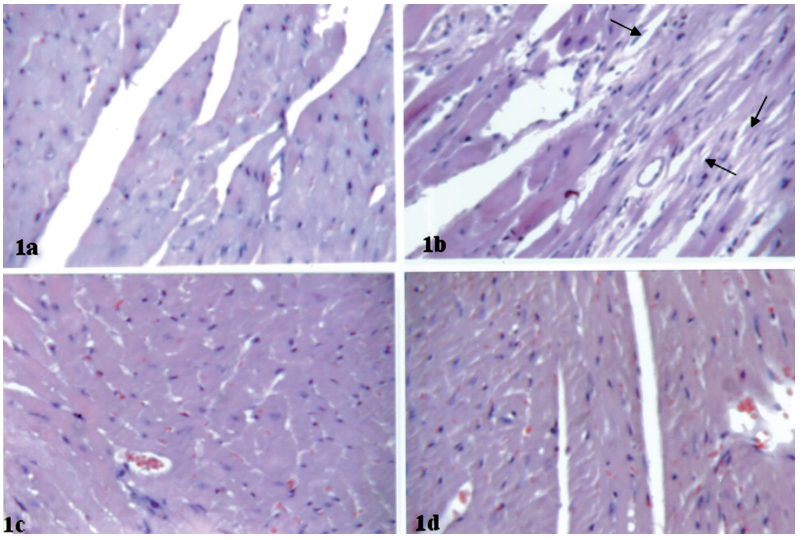

Fig. 1. The healthy myocardium of mice (a) and mice given CoQ10 (d) showed a typical structure, while hyperthyroidism resulted in disruption of linear array of cardiomyocytes (*arrows*) in heart of mice (b). CoQ10 administration improved this disorganization in hyperthyroid mice heart (c). Magnification for all figures stained with HE is 400.

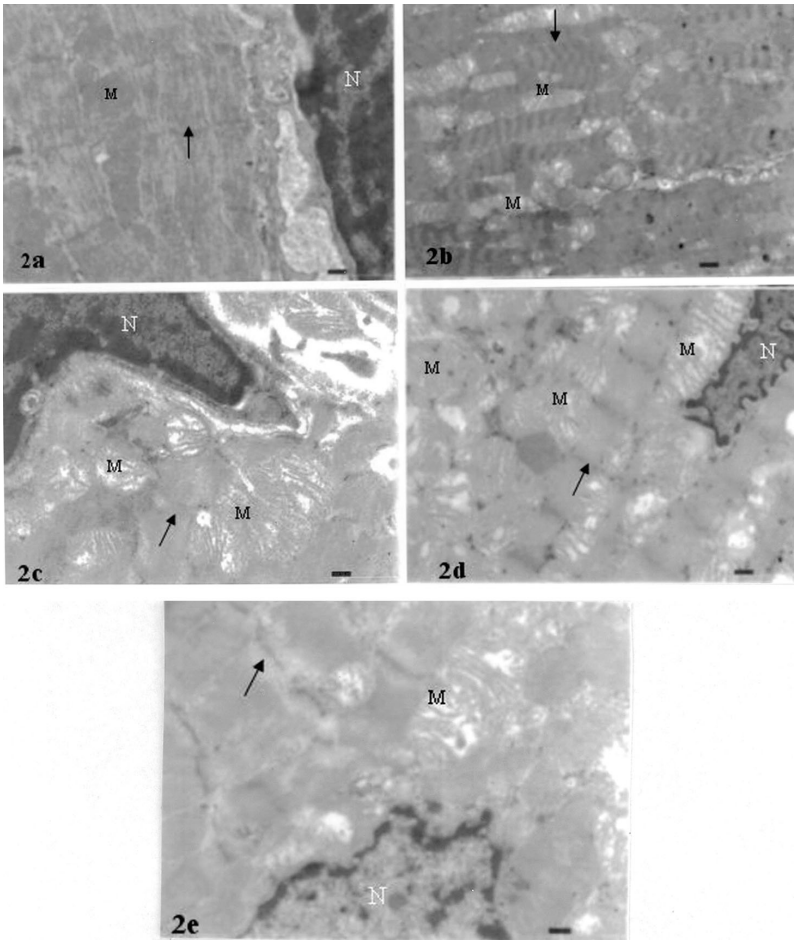

Fig. 2. It showed ultrastructure of myocardium from healthy mice (a) and hyperthyroid mice (b, c) as well as healthy (e) and hyperthyroid mice received CoQ10 (d). Fig. 2b presented general overview of hyperthyroid mice heart in low magnification. M: mitochondria; N: Nuclei of cardiomyocytes; →: Z line. Scales: 100 μm.

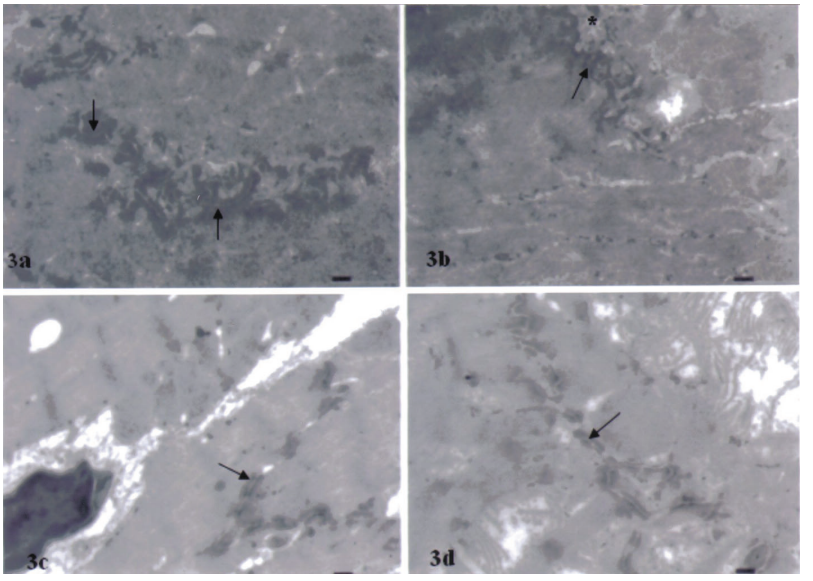

Fig. 3. Intercellular junctions (arrows) disorganized in hyperthyroid mice heart (b; * note the presence of wide spaces), while it showed characteristic aspect in the heart from healthy mice (a) and mice given CoQ10 (d). CoQ10 administration to hyperthyroid mice reversed structure of intercellular junctions to control images of junctions (c). Scales: 100 μm.

eNOS- and iNOS-IR were found in the myocardium of hyperthyroid mice heart (Figs. 4b and 5b). These IR for eNOS and iNOS were decreased in hyperthyroid mice heart after the administration of CoQ10 (Figs. 4c and 5c). However, iNOS-IR was intensive, when compared to the controls. The myocardium of healthy mice given CoQ10 displayed increased eNOS- and iNOS-IR, but not much more as the hyperthyroid heart (Figs. 4d and 5d).

Discussion

The present study demonstrated clearly a great degree of ultrastructural damage in the heart of hyperthyroid mice with T_4-induced. It suggested that CoQ10 administration could be an effective therapy in improving structural alterations and the regulation of eNOS and iNOS synthesis for the hyperthyroid heart as well.

Hyperthyroidism produces rapid cardiomyocytes hypertrophy with all subcellular components increasing in an orderly manner. Thyrotoxic myocardium has many myocytes showing considerable disorganization of sarcomeric myofilaments and big mitochondria with laceyappearence (17). Susceptibility to *in vitro* oxidative challenge and susceptibility to Ca^{+2}-induced swelling increased in mitochondria from hyperthyroid rat heart (32). Furthermore, the predominant appearance of myofibrillar disarray associated with disorganization of cytoskeletal proteins in addition to disorganization of intercellular junctions noted in the rat myocardium under thyrotoxicosis (7). The present structural alterations were consistent with the ultrastructural report of the other studies performed on hyperthyroid animal models and patients but differ from observations of Hu *et al.* (9), who reported the cardiac hypertrophy development and disorganization of intercellular junctions without myocardial degeneration.

Hyperthyroid stimulated a significant increase in heart rate, oxygen consumption and all systolic blood pressure. For this reason, hyperthyroid heart is obviously characterized by an increasing cardiac contractile performance, oxygen consumption and energy demand. In the present study, mitochondria increased in number. Hypertrophy of mitochondria and destructive structural changes in the contractile apparatus of the myocytes could be associated with excess of cardiac performance. Similarly, Ferreira *et al.* (7) remarked that myofibrillar disarray and disorganization of intercellular junctions might be due to higher mechanical wall stress and consequent higher metabolic demand. Alternatively, localization of numerous giant mitochondria among myofibrils might cause disorganization of myofibrils in the hyperthyroid heart of mice. The presented abnormality of intercala discs in hyperthyroid heart of mice as well as the other structural alterations would also agree with the proposal which these abnormalities originated from excessive performance can result in a loss of effective contraction force for further time (9).

The elevated cardiac performance can follow injury with oxidative and/or nitrosive stress in the heart under hyperthyroidism (2, 8, 21). Nitric oxide, particularly

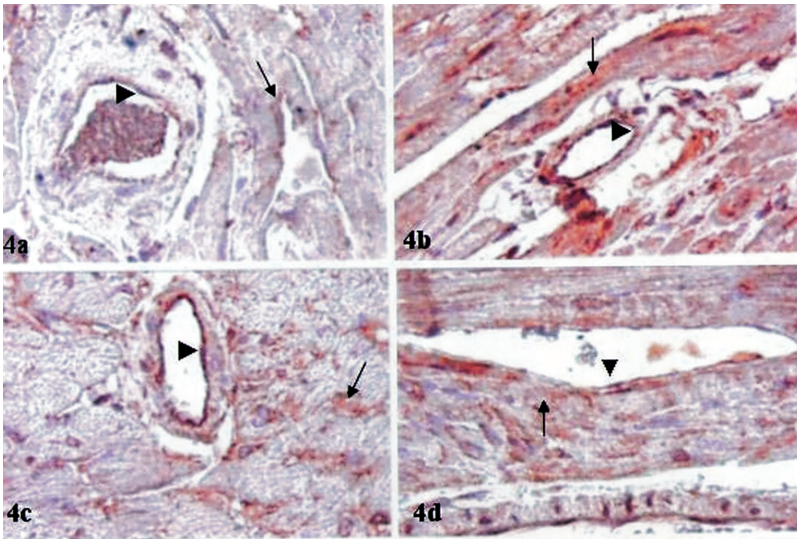

Fig. 4. eNOS-IR in all experimental groups; ▶: endothelial eNOS-IR; →: eNOS-IR in cardiomyocytes. Immunohistochemical localization of eNOS was found in the endothelial cells and cardiomyocytes of healthy mice heart (a). Elevated eNOS-IR exhibited in hyperthyroid heart (b) was decreased by CoQ10 administration to hyperthyroid mice (c). The myocardium of healthy mice given CoQ10 (d) displayed increased eNOS-IR when compared to controls. Magnification for all figures is 400.

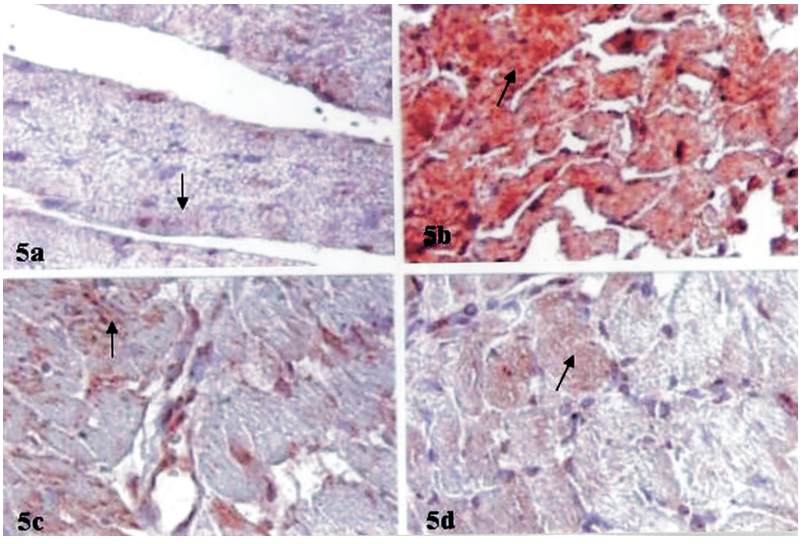

Fig. 5. Elevated iNOS-IR (*arrows*) in hyperthyroid heart (b), and hyperthyroid (c) and healthy heart (d) given CoQ10 when compared to controls (a). Elevated iNOS-IR in hyperthyroid mice (b) decreased in hyperthyroid heart treatment with CoQ10 (c). Magnification for all figures is 400.

derived from eNOS, can modulate mitochondrial respiration and tissue oxygen consumption in the whole body, heart, skeletal muscle, and kidney (19, 28). NO produced by iNOS is accompanied by elevated oxidative stress and could lead to peroxynitrite induced contractile impairment in hypertrophied or failing myocardium (30). NO and its potent oxidative derivate peroxynitrite *via* oxidation, hydroxylation and nitration involved in mitochondrial damage (27). Therefore, the eNOS and iNOS overexpression of heart from hyperthyroid mice can be reduced mitochondrial and the other structural damage. However, Rodriquez-Gomez and co-workers (26) considered that increased NO might play a protective homeostatic role in hyperthyroidism against the prohypertensive effects of thyroid hormone in rat heart. More recently, it is also suggested that NO is cardio-protective, antihypertrophic and antiapoptatic agent in cardiac-remodelling (29, 35). Cardioprotective

function of eNOS and iNOS for short-term and long-term defence, respectively, showed myocardial ischemia (3, 18). Obviously, NO level is very important and critical for structural and physiological integration of hyperthyroid heart. On the other hand, mitochondrial CoQ10 induced NO consumption in mitochondria (10). If this proposal is possible for NO of cardiomyocytes, the value of CoQ10 administration will be increased in hyperthyroid heart therapy. The present study demonstrated the improved structural alterations as well as decreased eNOS and iNOS-IR in heart of hyperthyroid mice given CoQ10. Moreover, hyperthyroid mice treated with CoQ10 showed increase eNOS and iNOS-IR of myocardium when compared to the controls, but a decreased eNOS and iNOS-IR when compared hyperthyroid animals. These results indicate that CoQ10 administration is quite useful for the hyperthyroid heart in question.

CoQ10 is a potent antioxidant and critical intermediate of the electron transport chain. Hyperthyroid patients displayed low serum levels of CoQ10 and congestive heart failure. CoQ10 administration daily for one week to these patients assessed the change positively in cardiac performance (31). Experimental evidence indicates that hyperthyroidism-induced heart dysfunction is a result of oxidative damage due to an increased production of reactive oxygen species (ROS). Venditti *et al*. (33) represented a link between oxidative challenge of mitochondria and their susceptibility Ca^{+2}-induced swelling during hyperthyroidism. This was also supported by the present results about the mitochondria in hyperthyroid mice heart. Furthermore, experimental studies showed an induced oxidative stress and reduced free radical scavengers in the heart of hyperthyroid patients and mice rendered hyperthyroid by T_4 (2, 8). Ferrara *et al*. (6) declared that acute exogenous treatment of CoQ10 protected the heart against oxidant-mediated injury in the rat. CoQ10 increases resistance of myocardium to oxidative stress by a direct antioxidant mechanism and/or indirectly due to increased protection of antioxidant (15, 16). Similarly, the other studies reported that reduced lesions in the ultrastructure of cardiomyocytes and depression of oxidative metabolism of mitochondria associated with increased antioxidant protection of myocardium after antioxidant administration, such as CoQ10, vitamin C and E (8, 14-16, 20, 34). In the present study, the probable reason for regressing of structural alterations in hyperthyroid mice heart given CoQ10 is the protective effect of CoQ10 as an antioxidant, using way/or ways above-described, besides reducing-effect eNOS and iNOS-IR.

The heart is a vital organ particularly poor in antioxidants and its postmitotic character makes more difficult the repair of tissue damage. Consequently, exogenous CoQ10 administration can be suggested for therapy of the hyperthyroid heart due to improving of structural disorders and regulation of eNOS and iNOS synthesis, despite of partially mitochondrial damage and hyperthyroidism.

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